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U.S. ARMY SOLDIER AND BIOLOGICAL CHEMICAL COMMAND

ECBC-TR-355

**DOMESTIC PREPAREDNESS PROGRAM:  
EVALUATION OF THE TRAVEL/R HCl™  
HAZMAT CHEMICAL IDENTIFIER**



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13. ABSTRACT (Maximum 200 words)  This report characterizes the chemical warfare (CW) and biological warfare (BW) agent detection characteristics of the TravelIR HCITM commercially available instrument from SensIR Incorporated (Danbury, CT). The TravelIR instruments were tested against HD, GB, GA, VX, and bio-simulant contained in various mixtures. This report is intended to provide the users concerned with CW agent detection an overview of the substance identification capabilities of the TravelIR.			
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## PREFACE

The work described in this report was authorized under the Expert Assistance (Equipment Test) Program for the U.S. Army Edgewood Chemical Biological Center (ECBC) Homeland Defense Business Unit.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

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## DOMESTIC PREPAREDNESS PROGRAM: EVALUATION OF THE TRAVELIR HCI™ HAZMAT CHEMICAL IDENTIFIER

### 1. INTRODUCTION

The Department of Defense (DOD) formed the Domestic Preparedness (DP) Program in 1996 in response to Public Law 104-201. One of the objectives is to enhance federal, state, and local capabilities to respond to Nuclear, Biological and Chemical (NBC) terrorism incidents. Emergency responders who encounter either a contaminated or a potentially contaminated area must survey the area for the presence of either toxic or explosive vapors. Presently, the vapor detectors commonly used are not designed to detect and identify chemical warfare (CW) agents. Little data are available concerning the ability of these commonly used, commercially available detection devices to detect CW agents. Under the DP Expert Assistance (Test Equipment) Program, the U.S. Army Soldier and Biological Chemical Command (SBCCOM) established a program to address this need. The Applied Chemistry Team (ACT), Aberdeen Proving Ground, MD, performed the testing. ACT is tasked with providing the necessary information to aid authorities in the selection of detection equipment applicable to their needs.

Reports of the instrument evaluations are posted in the Homeland Defense website (<http://hld.sbccom.army.mil/>) for public access. Instruments evaluated and reported since 1988 are listed in Appendix A.

In 2002, the evaluation of instruments continued using test items that were loaned to the DP program by their respective manufacturers. Viable candidate instruments were required to pass a pre-screening test. In exchange, the instruments were evaluated under the DP protocol revised to permit pertinent evaluations of the different instruments. Manufacturer representatives were permitted to take data during the evaluations to gain the maximum benefit from the testing. It has proven to be beneficial to the Domestic Preparedness Program and the device developer. Instruments evaluated in 2002 included:

- IMS 2000 from Bruker Daltonics GmbH, Switzerland
- RAID-M from Bruker Saxonia Analytik GmbH, Leipzig, Germany
- TravelIR from SensIR Technologies, Danbury, CT

Each of these evaluations will be reported separately. This report pertains to the evaluation of the TravelIR from SensIR Incorporated.

SensIR Technologies of Danbury, CT ([www.sensir.com](http://www.sensir.com)), markets the TravelIR HCI™ HazMat Chemical Identifier, a portable Fourier Transform Infrared (FTIR) analyzer, to the emergency response community ([http://www.hazmatid.com/Reference%20Corner/TravelIR\\_HCI.pdf](http://www.hazmatid.com/Reference%20Corner/TravelIR_HCI.pdf)). The manufacturer claims that the TravelIR HCI™ is being used for on-site identification of unknown white powders as well as common chemicals and toxic industrial chemicals in hazardous material spills. In situations where there is clearly a visible threat, the TravelIR can rapidly identify an unknown substance.

The TravelIR is not classified as a "detector" because it is not fitted with the gas cell for vapor detection. The instrument is considered as an "identifier." It is normally used in conjunction with other traditional detection equipment. Because most detectors are not capable of reliably identifying specific compounds but can give an indication that a threat may exist, the combination of technologies will provide the first responders with additional information to help them remedy the situation.

## 2. OBJECTIVE

The objective of this evaluation is to assess the general characteristics of the instrument and its ability to detect and identify the presence of CW agents and/or biological warfare (BW) agents in liquid or solid form. Prior protocol for evaluation needs to be modified for this testing. Because first responders frequently encounter unknown liquids or solid substances that may have been contaminated with CW or BW agents, this evaluation project was expanded to include these potential applications. Trace identification of substances in different mixtures is not considered the primary application for the TravelIR, but SensIR developed a user protocol to enable this potential of the TravelIR to be explored in this test.

## 3. SCOPE

This DP evaluation characterizes the CW liquid agent and BW agent detection capabilities of the instrument. Due to time and resources limitations, the CW agents used were limited to Tabun (GA), Sarin (GB), VX, and Mustard (HD). These were chosen as representative CW agents because they are believed to be the most likely threats. Test procedures were modified from the established DP Detector Test and Evaluation Protocol (see Appendix B) described in the Phase 1 test report.<sup>1</sup> Because the instrument is not a vapor detector, an exploratory approach was required to characterize the TravelIR's capabilities.

Another aspect of this evaluation was to investigate the ability of the instrument to identify biological spores. *Bacillus subtilis* var. *niger* (BG) was selected as a simulant for BW agents. This was done by mixing BG with various other substances and testing the mixtures with the TravelIR. The bio-simulant testing protocol was used to investigate the degree to which the TravelIR instrument can detect biological spores and to provide preliminary data regarding response and sensitivity in the presence of a sample matrix. Preparation of the test matrices for the CW agent and Bio-simulant testing followed the procedure outlined in Appendix B. The percentage concentration can be the percentage of the mixture in: weight to weight (solid in solid), weight to volume (solid in liquid), volume to weight (liquid in solid), or volume to volume (liquid in liquid) using the neat compounds. Liquid density was considered where appropriate. The concentration numbers appearing in this report should be treated as "approximate" due to the very small amounts of sample that were used in the serial dilutions.

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<sup>1</sup> Longworth, Terri L. et al *Testing of Commercially Available Detectors Against Chemical Warfare Agents: Summary Report*, ECBC-TR-033, U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 1999, UNCLASSIFIED Report (AD A364 123).

## 4.

#### DESCRIPTION OF THE TRAVELIR INSTRUMENT

The TravelIR is a FTIR spectrometer that can be used to identify unknown solids, powders, pastes, gels, and liquids (see Figure 1). The technique involves placing a sample on top of a diamond crystal (which has a high refractive index) embedded in a stainless steel disk, called the DuraDisk. An infrared (IR) beam from the spectrometer passes through the crystal and penetrates slightly into the sample to permit analyzing strong IR absorbing solutions, such as emulsions or aqueous solutions. The technique can also be useful for solid samples. The process is non-destructive to the sample or the instrument. Unknown samples are identified by comparison with reference database libraries.

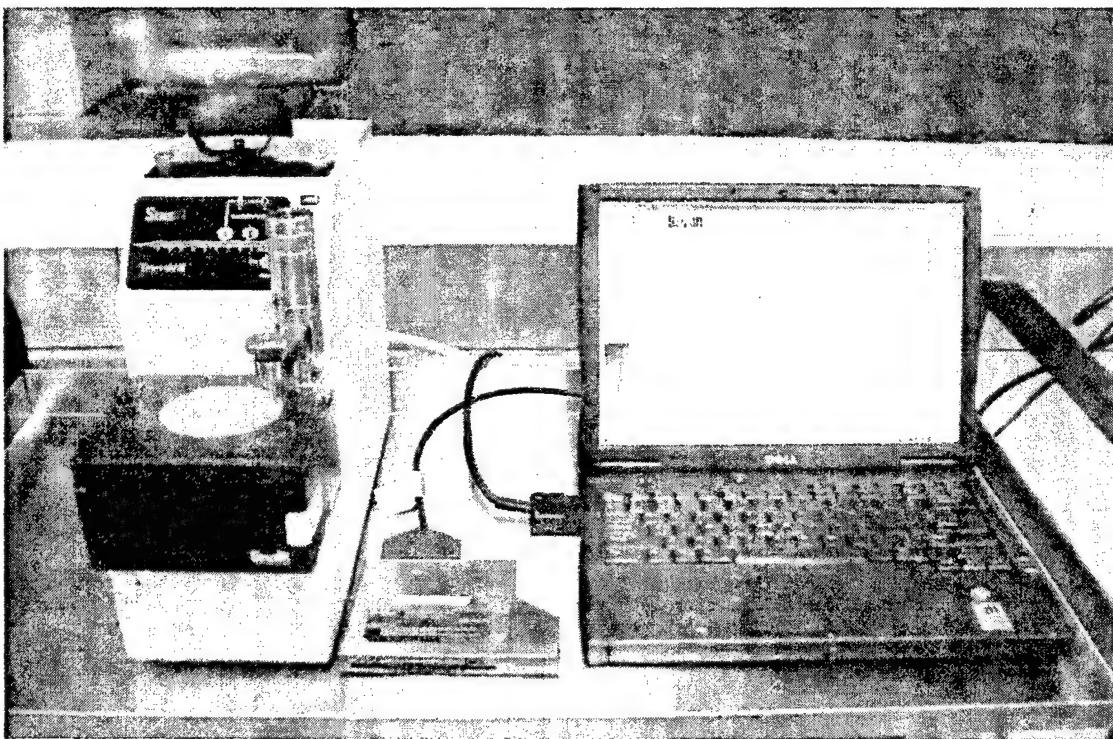


Figure 1. TravelIR from SensIR Technologies

In Figure 1, a "single reflection" disk is installed and the system is connected with a laptop computer. The "single reflection" disk is mainly used for solid samples. There are three different variations of stainless steel DuraDisk available (see Figure 2). The "three reflection" disk, not shown, provides flexible analysis on a variety of samples, either solid or liquid. The "nine reflection" disk is used for liquid samples only. The "volatile cover" is a clear plastic cover to be placed on top of the sample to minimize sample loss due to high volatility during an analysis. The "safety solid sampler" was developed to permit remote loading of a solid sample. The sample is sealed within an O-ringed area together with a spring loaded pressure arm to permit safe handling of potentially toxic sample to be analyzed by the instrument. Good contact between the sample and the crystal is important. For solid samples, the pressure arm is used to apply controlled force to the sample to achieve better contact with the crystal surface. The TravelIR is said to be operable between 0 and +40 °C.

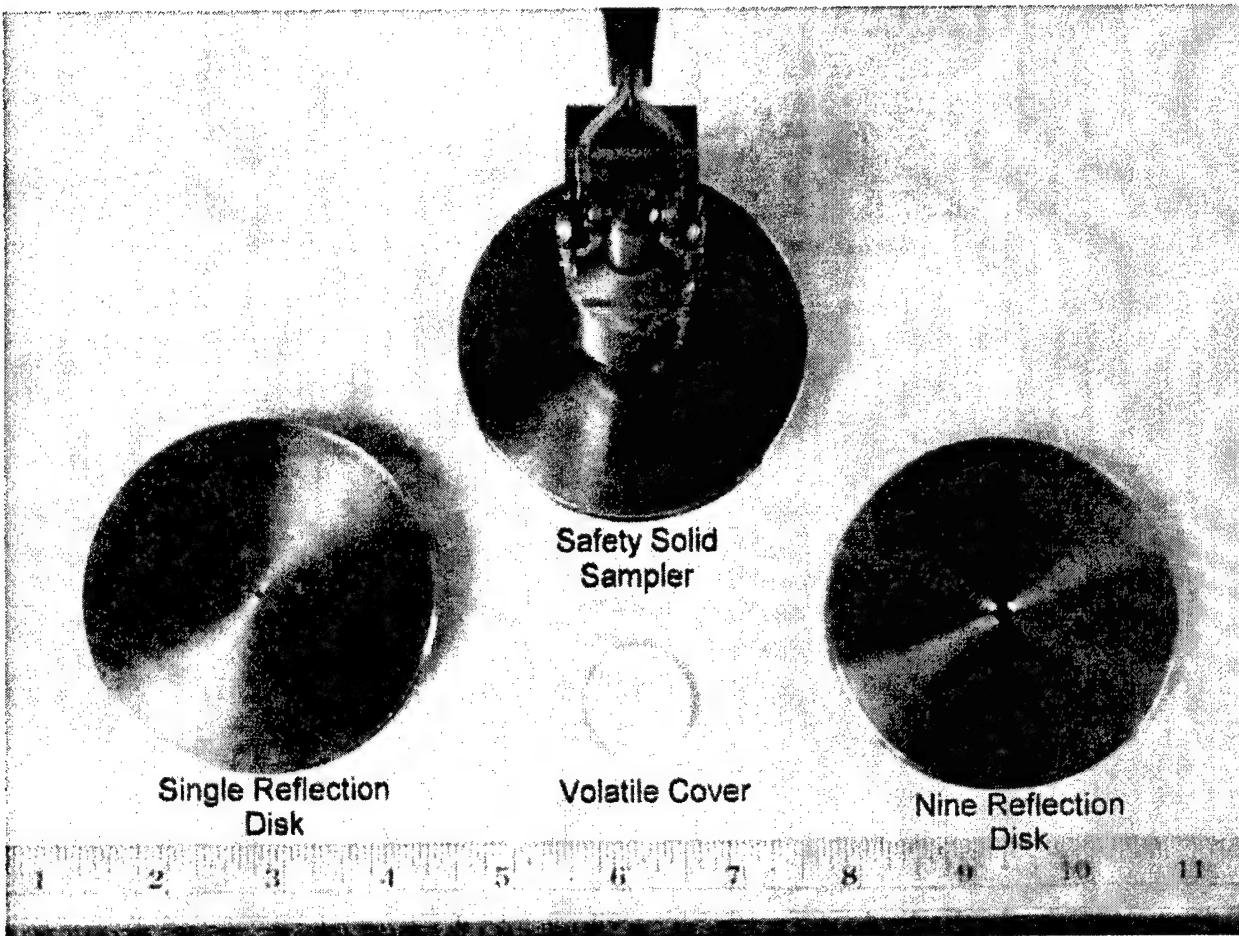


Figure 2. Sampling Accessories

The basic TravelIR unit dimensions are: 16.5" in length; 5.2" in width; and 12" in height. It weighs approximately 26 lb and must be connected with a laptop computer for its normal operation.

The instrument can be operated using 110 Vac, battery power supplies, or 12 Vdc automobile battery power. It requires a computer with a 200MHz CPU and 32 MB of RAM or above to operate. The system is packed in a hard carrying case for transportation.

## 5. TESTING PROCEDURES

### 5.1 Sample Preparation for Analysis.

Liquid samples were prepared by volume ratio between the chemical warfare agents (CWA) and the substrate (i.e., the solvent or the carrier). After obtaining the instrument background on each trial, a 5- $\mu$ L sample was placed onto the nine-reflection DuraDisk well for analysis. For highly volatile samples (e.g., GB samples), a volatiles sample cover was used to

prevent sample evaporation. The instrument was set to collect the 60 spectra and conduct a library search for spectrum identification (approximately 60 sec). A list of closely matched spectra along with their identification and respective quality value was produced. Quality value is an indication of how close the sample spectrum matches the spectrum of each of the identified substances.

On neat materials, the identification quality value is usually in the high 90s. Higher quality values indicate more closely matched spectra. However, when the substances exist in a mixture, the quality of match becomes significantly altered due to the complex spectrum from the mixture of spectra. Usually, the identification list will show up with the major components. Often, the spectrum is overly complex. Then, the identification list could come up with substance names completely different than what the sample may contain, following the routine protocol provided by the *TravelIR* manual. Fortunately, the instrument includes software to permit spectra subtractions of the top hits using the Gram software and the following analysis protocol. Such a subtraction routine can then provide the "proper" identification, providing that the concentration of the constituent of interest exists in sufficient quantity.

Solid samples were prepared by weighing, and liquid samples were prepared by measuring the liquid volume, taking density into account, to achieve the test mixture percentage. Serial portioning for subsequent mixtures yielded the different concentrations. A small quantity of the liquid-solid mixture or solid-solid mixture was placed on the single reflection diamond Attenuated Total Reflection (ATR) sample disk, and a nominal 25-lb force was applied to the sample via the polished end of the stainless steel rod supplied with the *TravelIR*, to effect good sample contact, for the analysis.

## 5.2 Analysis Protocol.

An analysis protocol was developed, with a representative from SensIR at the U.S. Army Edgewood Chemical Biological Center (ECBC), in a separate Test Service Agreement. The protocol was necessary to expand the usefulness of the *TravelIR* toward CWA detection when the agent exists in a solid mixture or in solution. A nine- reflection diamond ATR sample disk was used in the analysis of all liquid mixtures, where a 5- $\mu$ L aliquot of the liquid sample was used. For volatile mixtures, a volatile cover was placed above the sample to minimize sample evaporation. The single reflection diamond ATR sample disk was used in the analyses of all liquid-solid (e.g., GB in silica) or solid-solid (e.g., BG spores with powdered milk) mixtures.

*TravelIR* utilizes QualID version 1.53 control software for the collection of the IR spectral data. The IR spectral database search function was accomplished using ThermoGalactic SpecID™ version 3.01 software. The format of the spectral data files and libraries are the same for both software packages. Several IR spectral libraries were loaded into the system and searched in each analysis. A description of the libraries is tabulated in Table 1, below.

**Table 1. IR Spectral Libraries Used in the Analysis of CWA -Interference Mixtures**

<b>Library File Name</b>	<b>Description</b>	<b>No. of Entries</b>
SensIRcc.lib	Common Chemicals, mostly organic chemicals	1896
4 <sup>th</sup> wmd cst.lib	WMD spectral data	27
APG-ACT.lib	Reference Data collected for the DP evaluation and this study	15
ATR-v01	Ichem ATR spectral library, mostly organic chemicals	1000
SensIRDp.lib	DEA controlled narcotic drug precursors/solvents	44
SensIRExplosives.lib	Common explosives	31
SensIRfo.lib	Narcotics Drug Library from IL state police	453
SensIRtx.lib	Toxic chemicals from the NIOSH priority list	72
SensIRwp.lib	Commonly encountered white powders	36

The libraries were searched using a normalized dot product, first derivative, and correlation algorithm. The Hit Quality Index (HQI) is a similarity metric computed from the sample spectrum and each library spectrum. The 20 library spectra that are most similar to the unknown are tabulated on the search "hit list." An HQI of zero (0) indicates a perfect match; therefore, the lower the HQI, the more closely matched are the unknown and library spectra. Prior to the HQI calculation, the first derivatives of the sample spectrum and library spectrum are computed and mean centered.

The HQI determines the search reported quality value. A lower HQI yields a higher quality value of the spectra match of the sample to the spectrum of the compound identified from the libraries. Higher quality value indicates with more certainty what the sample could be. Usually, a quality value of >80 can be considered preferable.

To facilitate the agent in a substrate (e.g., agent mixed with floor wax) mixture analyses, reference spectra of the substrate substances were collected and added to the APG-ACT (Aberdeen Proving Ground-Applied Chemistry Team) library so that they were available for spectrum subtraction. The CWAs that contain a phosphonate group interact strongly with water through hydrogen bonding. This interaction shifts the CWA absorption bands significantly due to perturbation of the electric field of the molecule by the hydrogen bonding interaction. Thus, for aqueous systems, "bound" reference data was collected and also added to the APG-ACT library. The bound reference data was collected by recording the spectrum of the phosphonate type of CWA in water and digitally removing the water contribution before adding the reference data to the library.

Identifying mixture components using IR search techniques is a two-step process. In the first run through search, one of the components of the mixture will assume the top (or a high) position on the "hit list." A major component of the mixture is identified. The software then allows a digital compensation where the contribution of the component is "subtracted out" from the mixture spectrum. After compensation, the residual spectral signature is searched against the same libraries and the graphical comparison repeated.

### **5.3      CWA Testing.**

#### **5.3.1      Minimum Detectable Concentration.**

To establish the minimum CWA detectable concentration of the TravelIR, solutions of different agent concentrations in isopropyl alcohol were made. A 5- $\mu$ L sample was placed onto the cavity well of the nine reflection sample disk. The alcohol was allowed to evaporate, while watching the sample spectrum displayed for disappearance of the characteristic alcohol peak, before spectral analysis of the residual CWA of interest was performed. The MDL determined represents the minimum concentration of the solution required to generate a correct detection/identification using the evaporation technique.

#### **5.3.2      CWA Detection in a Substrate.**

A small quantity of the liquid-solid mixture was placed on the single reflection diamond ATR sample disk, and a nominal 25-lb force was applied to the sample via the polished end, stainless steel rod supplied with the TravelIR. All spectral data were recorded at 4 cm<sup>-1</sup> spectral resolution using 60 averaged scans.

Because of the large proportion of the substrate in the mixtures, the initial result from the automated spectral search usually identified the "substances" of the mixture as something altogether different. Therefore, to detect the presence of CWA in mixtures, it was necessary to subtract the predominant substrate spectrum from the spectrum of the total mixture following the analysis protocol given in Paragraph 5.2.

### **5.4      Bio-Simulant Testing.**

For BG testing, a background spectrum averaged over sixty scans was recorded from the clean sensor element prior to recording the data from each sample. A small quantity of the liquid-solid mixture was placed on the single reflection diamond ATR sensor, and a nominal 25-lb force was applied to the sample via the polished-end, stainless steel rod supplied with the TravelIR (apply pressure to "7" on the LED display). All spectral data were recorded at 4 cm<sup>-1</sup> spectral resolution using 60 averaged scans. Absorption bands near 3300 cm<sup>-1</sup> (NH), 1640 cm<sup>-1</sup> (amide I), 1530 cm<sup>-1</sup> (amide II), and 1250 cm<sup>-1</sup> (amide III) indicate protein and therefore, biological material. Further confirmation of biological identification was provided by a spectral search against the reference library and the results were noted. The procedure was repeated for each mixture.

#### **5.4.1      Standards for Detection.**

The BG spores, Lot 10-88, in dry powder form (originally manufactured for Dugway Proving Ground by Bioferm) were used for testing of the TravelIR. Previous studies were performed for sample integrity and enumeration with a viable spore count-to-weight ratio of approximately  $3.6 \times 10^{12}$  colony forming units/gram (cfu/gm).<sup>2</sup> The BG was tested "neat" and

<sup>2</sup> Larsen, L. D., and Harper, B. G., 1988. Final Report on Fermentation and Maintenance of Simulant Stocks. Prepared for Life Sciences Division, Material Test Directorate, U.S. Army, Dugway Proving Ground, Utah.

diluted in each of the following matrices: silica, non-fat dry milk (NFD Milk), floor wax, aqueous film-forming foam (AFFF), and diesel fuel. Each matrix was tested in pure form (i.e., "neat") and submitted to the spectral library in Grams as a formal matrix blank. Initial BG dilutions were prepared using 1 g of dry material to 1 g of dried matrix (silica, NFD milk) or 1 mL of matrix (floor wax, AFFF, diesel fuel). Subsequent serial 1:2 dilutions were initially prepared to a concentration of 1:256 (0.39% BG). Further dilutions (to 1:4096, 0.02%BG) were prepared for NFD milk and AFFF in effort to determine the detection limit. All spore preparation and matrix combination work was conducted in a biosafety cabinet, certified at BSL 2.

The TravelIR FT-IR device uses multiple software systems for detecting and analyzing IR spectra. The primary database search tool, QualID Software, automatically matches the test spectrum with reference spectra from a database library and reports results based on set parameters. Initially QualID reported only hits with a "quality" score of 80% or greater due to method parameters (set by SensIR). After several samples were analyzed, it was evident that the software algorithms on the instrument were configured to reduce or eliminate reporting of minor, and possibly distracting, components contained in the test sample. Therefore, the quality threshold value was dropped to zero, reducing software "sensitivity" by allowing the inclusion of minor sample components in the resulting output. Consequently, the instrument reported a greater number of hits (10) for each sample.

Spectra of BG spores were added as "BG neat" to the QualID database software library. For detection probability, a "hit" identifying "BG neat" in the top ten of QualID was considered a positive result. When no correct hit was obtained in the top ten of QualID search, spectral subtract and a second search was performed within the Grams and Spectral ID (SpecID) programs, respectively. The spectral subtract function involved subtracting the spectrum of the known matrix from the test spectrum. The resulting spectrum was compared with the database library through a second search engine. This search, done in SpecID, returned a list of 20 best matches and reported a quality score that required manipulation for comparison with quality results from QualID [(i.e., quality = (1- SpecID quality) x 100)]. A top twenty hit of "neat BG" of any quality was considered a positive result for the purpose of this report. Sample preparations were tested in triplicate. Two out of three positive results were required to designate the identification as successful with either of the search algorithms.

#### 5.4.2 BG Detection in a Mixture.

In an attempt to determine detection limits with spectral subtract method, much lower concentrations of BG were prepared and tested than required by the test agreement. Higher concentrations of BG were not tested for NFD Milk and Floor wax (marked "NT" in Table 4) due to the instrument's ability to detect lower concentrations in these matrices. It was surmised that positive results for low dilutions and "neat" sample indicated that these concentrations would have a positive result as well.

## 6. RESULTS AND DISCUSSION

The TravelIR demonstrated repeatability during the evaluation. Its repeatability permitted a wider spread of tests rather than more repetitions. Minimal relative humidity (RH) and temperature effects were expected because of the nature of this testing protocol (i.e., the computer algorithm automatically subtracts water after background is set for the instrument) which was done at least daily. Thus, in lieu of RH/temperature testing, the evaluation focused on developing a user protocol to make the instrument more useful to the responders.

### 6.1 Minimum Detection Level.

#### 6.1.1 BG Detection.

Table 2 is a summary of the numerical detection limit estimates determined by each of the software programs (Qual ID and Grams SpecID). Exact detection limits were not determined for four of the five matrices due to the low detection level seen with the SpecID program.

Table 2. Minimum Detectable (%) Concentration of BWA Simulant (BG)

Matrix	QualID	Grams (SpecID)
Silica	25	< 0.19
NFD Milk	0.39	< 0.02
Floor Wax	25	1.56
Aqueous Film Forming Foam (AFFF)	50	< 0.39
Diesel Fuel	25	< 0.02

#### 6.1.2 CWA Detection.

The minimum amount of CWA the TravelIR detected was determined from solutions of different agent concentrations in isopropyl alcohol (IPA). A 5- $\mu$ L sample was placed onto the cavity well of the nine reflection sample disk. Initially, by observing the disappearance of the solvent peaks, the alcohol was allowed to evaporate before spectral analysis of the residue CWA of interest was performed. The minimum residual amount of agent the TravelIR could detect from a 5- $\mu$ L sample (using nine reflection disk and solvent evaporated) is summarized in Table 3.

Table 3. Minimum Detectable Residual from CWA Concentrations (%) in IPA

Agent	CWA Concentration (%) in IPA (before solvent evaporation)
GA	0.625
GB	5.0
VX	0.016
HD	0.25

## 6.2 Detection in Mixtures.

### 6.2.1 BG Spores in Mixtures.

The TravelIR FT-IR device detected spores in all of the test matrices. Table 4 lists the qualitative results of BG spores mixed in different substrates, from each of the individual replicates tested, for every BG concentration tested. See Appendix C for more detailed data tables and graphs from each of the five matrices tested.

**Table 4. Qualitative Results Compilation of Spectra from BG Matrices**

Dilution	BG %	Silica Dust			NFD Milk			Floor Wax			AFFF			Diesel Fuel		
Neat	100	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
1/2	50	Y	Y	Y	NT	NT	NT	NT	NT	NT	Y	Y	Y	Y	Y	Y
1/4	25	SS	Y	Y	NT	NT	NT	Y	Y	Y	SS	SS	SS	Y	Y	Y
1/8	12.50	SS	SS	F	NT	NT	NT	Y	SS	SS	SS	SS	SS	SS	SS	SS
1/16	6.25	SS	SS	SS	NT	NT	NT	SS	SS	SS	SS	SS	SS	SS	Y	SS
1/32	3.13	SS	SS	SS	NT	NT	NT	SS	SS	SS	SS	SS	SS	SS	SS	SS
1/64	1.56	SS	SS	SS	NT	NT	NT	N	SS	SS	SS	SS	SS	SS	SS	SS
1/128	0.78	F	SS	SS	Y	Y	Y	N	N	SS	SS	SS	SS	SS	SS	SS
1/256	0.39	SS	F	F	SS	Y	Y	N	SS	N	SS	SS	SS	SS	SS	SS
1/512	0.20	SS	SS	F	Y	SS	Y	NT	NT	NT	SS	SS	SS	NT	NT	NT
1/1024	0.10	NT	NT	NT	SS	SS	Y	NT	NT	NT	SS	SS	SS	NT	NT	NT
1/2048	0.05	NT	NT	NT	SS	SS	SS	NT	NT	NT	SS	SS	SS	NT	NT	NT
1/4096	0.02	NT	NT	NT	SS	Y	SS	NT	NT	NT	SS	SS	SS	NT	NT	NT

"Y" means BG was top ten hit

"SS" means BG detected after spectral subtract

"N" means no BG detected

"F" means file lost

"NT" means not tested

"Neat" means 100% BG (i.e.,  $3.6 \times 10^{12}$  cfu/gm)

QualID was able to identify samples with a correct match among the top ten hits when the samples were of 50-25% BG, with the exception of BG in NFD Milk. This matrix allowed detection of BG to a concentration of 0.20% in NFD Milk. Using the Grams program provided detection that is more sensitive by allowing the subtraction of "obvious interferents," referred to here as "neat" matrix, from the initial spectrum followed by a manual search in the SpecID program. Spectral subtract allowed for the detection of BG concentrations of 0.4% or lower with each of the tested matrices with the exception of floor wax.

## 6.2.2

CWA in Mixtures.

Table 5 is a summary table of results using agents GA, GB, VX, and HD in several different media. These media represented the CWA detection potential under different conditions in a single component solution, in a multi-component aqueous solution (AFFF, Spray 9, and floor wax), non-aqueous medium (diesel fuel), and adsorbed to a solid (silica flour). The spectrum of each CWA in water, with the spectrum of the water removed by spectral subtraction, was added to the library. The analyses of solid or silica mixtures are complicated due to the contribution of anomalous dispersion and concomitant frequency shifts for the strong SiO<sub>2</sub> stretching frequency, resulting in a significant silica signal in the residual spectrum even after subtraction.

Table 5. Minimum Detectable Concentration (%) of CWA in Mixtures

Substance	Agent Concentration (%)			
	GA	GB	VX	HD
Water	10	5	1	10 *
AFFF	2.5	2.5	Failed at 5	50 *
				10 **
Spray 9	10	10 *	5 **	Failed at 10 *
			5 ***	10 **
Floor Wax	5	2.5	5 *	Failed at 10 *
Diesel Fuel	10 *	2.5 ***	2.5	25 ***
Silica Flour	10 ***	10 ***	5	10

\* Analyzed from top layer of insoluble system

\*\* Analyzed from bottom layer of insoluble system

\*\*\* 3rd on list of identified compounds after spectral subtraction

Tabun, GA, forms two phases with diesel fuel. The diesel (top) layer was analyzed. The lowest concentration analyzed was 10 % v/v for the GA/diesel fuel mixture. Tabun is soluble in water and in aqueous systems. These identifications relied upon reference data for "bound" GA. The spectrum of GA in water was recorded, and the water was removed by digital subtraction. This water compensated data was added to the spectral library and reflects GA that interacts through hydrogen bonding with the water solvent.

Although GB is not completely soluble in diesel fuel at a 20% v/v level, it does dissolve at a 10% v/v level. The minimum detectable concentration of GB in diesel fuel is at 2.5% v/v level. Although GF was not a test compound, its spectrum is included in the instrument's spectral library. Several mixtures of GB in diesel showed higher spectral quality value for cyclo-sarin (GF) than GB itself. Perhaps this is due to the structural and spectral similarity of these two agents and incomplete compensation in the C-H (carbon-hydrogen) stretching region. Given the volatility of GB, the final concentration is suspect. The higher minimum identifiable quantity (MIQ) or minimum detectable concentration of GB in water

relative to GB in the other aqueous systems (i.e., AFFF, Spray 9, and Floor Wax) may be due to loss of GB through evaporation and a final concentration lower than 5% v/v. Certainly the absorbance of GB is smaller than expected for this concentration.

The agent, VX, is sparingly soluble in aqueous systems at approximately 3% at ambient temperatures and pressures. Aqueous layers were sampled as saturated solutions at the higher concentrations to achieve identification after the routine subtractions. At higher concentrations, two phases are evident in aqueous systems, and the phases can be sampled separately. The MIQ for VX for water was 1% v/v. The VX agent was identified in other aqueous systems at the 5% level (phase separated).

Mustard, HD, is immiscible in aqueous systems, and HD is sampled as a separate phase. With the density of HD being 1.268 g/mL at 25 °C, the bottom layer is the suspected HD layer. However, HD is identifiable in the top aqueous layer at concentrations greater than a 10 % v/v level after spectral subtraction. Positive HD identification is obtained when the suspected HD bottom layer is sampled. Analysis of mixtures below 10 % v/v was not tried. The MIQ for the HD/ diesel mixtures was relatively high, reflecting a lack of specificity for this material. The HD was identified in the presence of silica (a dust simulant) at a MIQ of approximately 10%.

Some work was performed using chloroform extractions. For example, VX is very soluble in chloroform and thus can be extracted from the aqueous layer. A sample of the extract in chloroform was allowed to evaporate leaving VX on the diamond ATR sensor for analysis. Positive identifications to the 1% level in water and 5% level in floor wax were achieved using a chloroform extraction procedure. Similarly, CWA in silica flour can be extracted with a small amount of chloroform or other similar solvents to achieve positive identification.

Appendix D is the extraction protocol developed by SensIR Technologies as the result of this evaluation recommendation. The procedure follows the similar procedure tried with successful results. It is attached for users to follow to expand the usefulness of the instrument during their missions.

## 7. CONCLUSIONS AND RECOMMENDATIONS

The TravelIR can identify neat substances readily and reliably with reproducibility. However, due to physical laws, identification of a substance in a mixture is considerably more difficult. The complex spectrum from the mixture often leads to erroneous search results. Because unknown chemical warfare agents (CWA) found during an incident could potentially be mixed with dust or a liquid base, positive identification of such a material is important. Therefore, this evaluation explored those possibilities.

Generally, positive identification results were obtained with concentrations of CWA occurring in the 1-10% concentration range. Positive identification is defined as the top hits with quality value of >80 after the spectra subtraction routine. Where the CWA is not dissolved in a liquid, the CWA layer is readily identified correctly. The problem becomes much

more complex when the CWA is dissolved in or interspersed with the different substances. Therefore, the TravelIR should be used in conjunction with other approved CWA detectors. If the CWA detector is negative for agent, the TravelIR is well suited to identify the substances present.

If the solvent containing the agent is volatile and its evaporation leaves behind a concentrated sample of CWA, the TravelIR can readily identify the agent. This finding led to the chloroform extraction technique tried on VX mixtures. The findings were encouraging. Perhaps, by implementation of a simplified extraction process, the TravelIR would be much more useful for the identification of potential CWAs in a mixture.

The TravelIR instrument is found to be sensitive to biological material [*Bacillus subtilis* var. *niger* (BG spores)].<sup>\*</sup> The instrument is able to detect spores when mixed in the presence of several matrices, even when these matrices contain organic compounds. It detected BG spores well below initially expected levels when using the "spectral subtract" function in the Grams<sup>TM</sup> software. As detectable concentrations were much lower than expected in Grams<sup>TM</sup>, a detection limit was only determined for floor wax. The complex composition of floor wax created greater interference at critical wavelengths for Bio-detection thus decreasing the instrument's ability to detect spores mixed with this matrix. Further testing is required to determine detection limits in the remaining four matrices by the spectral subtract method (Grams<sup>TM</sup>/SpecID).

It is recommended that further investigation, testing, and cataloging of other potential interferents, such as components used for weapons, propellants, and naturally occurring environmental compounds be pursued. Also, a database library of reference spectra should be compiled for a variety of microbiological organisms under differing environmental stresses (e.g., grown on multiple culture media or various temperatures) and preparations (e.g., live, heat- or uv-killed).

It is further recommended that a simple extraction procedure be developed and incorporated in the user's manual to expand the ability for TravelIR to accurately identify the existence of CWA in a mixture. Such a technique might permit the instrument to correctly identify the suspected CWA down to the Minimum Detectable Level (MDL) as determined in this report.

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\* *Bacillus atrophaeus* formerly, *Bacillus subtilis* var. *niger*, formerly *Bacillus globigii*.

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## APPENDIX A

### LIST OF DETECTION DEVICES EVALUATED

#### Since the Beginning of This Test and Evaluation Program

- MiniRAE plus from RAE Systems, Incorporated (Sunnyvale, CA)
- Passport II Organic Vapor Monitor from Mine Safety Appliance Company (Pittsburgh, PA)
- PI-101 Trace Gas Analyzer from HNU Systems, Incorporated (Newton, MA)
- TVA 1000B Toxic Vapor Analyzer (PID and FID) from Foxboro Company (Foxboro, MA)
- Draeger Colorimetric Tubes (Thioether and Phosphoric Acid Ester) from Draeger Corporate (Pittsburgh, PA)
- Photovac MicroFID detector from PerkinElmer Corporate (Wellesley, MA)
- MIRAN SapphIRe Air Analyzer from Foxboro Company (Foxboro, MA)
- MSA Colorimetric Tubes (HD and Phosphoric Acid Ester) from Mine Safety Appliances Company (Pittsburgh, PA)
- M90-D1-C Chemical Warfare Detector from Environics OY, Finland
- APD2000 Detectors from Environmental Technologies Group, Incorporated (Baltimore, MD)
- SAW MiniCAD mkII from Microsensors Systems, Incorporated (Apopka, FL)
- UC AP2C Monitor from Proengin Incorporated, France
- ppbRAE Photo-Ionization Detector from RAE Systems, Incorporated (Sunnyvale, CA)
- SABRE2000 detector from Barringer Technologies, Incorporated (Warren, NJ)
- CAM (Type L) from Graseby Dynamics Ltd., UK
- VaporTracer System from Ion Track Instruments, Incorporated (Wilmington, MA)
- HAZMATCAD from Microsensor Systems, (Apopka, FL)
- GC-FPD/MSD with Dynatherm System from Agilent (Columbia, MD)
- Scentoscreen GC from Sentex Systems, Incorporated (Ridgefield, NJ)

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## APPENDIX B

### TEST PROTOCOL FOR CHEMICAL WARFARE AGENTS DETECTION, EVALUATION OF COMMERCIALLY AVAILABLE DETECTION DEVICES (TRAVELIR-HCI™ HAZMAT CHEMICAL IDENTIFIER)

This evaluation is designed to characterize the CW agent identification capability of the instrument. The agents used include Tabun (GA), Sarin (GB) and Mustard (HD) similar to those for vapor detectors. These are chosen as representative CW agents because they are believed to be the most likely threats. The VX is added to the list because of its low volatility, and most likely it will exist as liquid droplets.

The minimum concentration level (Minimum Detectable Level, MDL) for each volatile CW agent selected, where repeatable positive identification is obtained, is determined by using a 20- $\mu$ L sample of the lowest detectable concentration of agent in solution. The nine-reflection diamond ATR (Attenuated Total Reflection) disk is used for this experiment. The MDL is determined at ambient temperature and relative humidity (RH) conditions. The MDL for this testing is determined by "Minimum Detectable Residual from CWA Concentrations (%) in isopropyl alcohol (IPA)."

The instrument capability will be determined for each of the CW agents at ambient and high (>80%) and low (<20%) RH (achievable range by the test chamber) using one instrument placed inside an environmental chamber. Similarly, tests will also be conducted at temperature extremes (0 and +40 °C) to investigate the effects of humidity and temperature on the detector's behavior. Note: There shall be no defined humidity condition at 0 °C (record the RH of the chamber during testing) and the humidity at 40 °C will be at approximately 60% to simulate close to the worst real world situation in terms of moisture content.

Observe the effects of potential interference with commonly found substances mixed with the agents to assess the ability of the detector to identify and discriminate the existence of CW agents. The nine-reflection diamond ATR disk will be used for this evaluation. The interfering materials will be added to the reference database and stripped from the analytical data using digital subtraction techniques. This testing will be done at ambient temperature and RH conditions.

The ability to detect and identify the neat substance in the laboratory under clean, controlled conditions is insufficient to determine whether the device is useful at less than ideal conditions. The units will be tested with CW agents dissolved (mixed) at concentrations from 1% to 5% in several selected common potential interference substances to determine if the instrument can identify the CWA using trace overlays and spectral searching on digital "stripped" residues. Because it is not possible to cover every substance, the following have been selected for the trials:

- Aqueous Film Forming Foam (AFFF)
- Spray 9 Household cleaner
- Floor Wax
- Diesel fuel
- Fine Dust

If the above substances' spectra have not been included in the instrument's existing internal library, such a library will be constructed to enable potential overlay. The library may include spectra of the CWA and substance mixtures. Overlaying the spectra permits subtraction of the potential interfering trace to yield more reliable identification of unknowns (i.e., CWA). Overlaying will not be necessary if the library search correctly identifies the suspect CW agent with a library quality value (Q value) >80 on the library hit list. Such tests will demonstrate the instrument's ability to discriminate CWA from potential interfering components in the suspected contamination.

Because it is impractical to test detection systems with toxic CW agents in open air, potential interfering substances are tested in the laboratory at controlled exposure levels. Field tests of the system will not be conducted.

General observations made during the test program are sufficient to determine the relative stability and reliability of the instrument. Abnormalities and problems will be recorded. The extent of this type of evaluation is limited to the above mentioned characterization criteria. Test conditions will not exceed the instrument's functional potential as given by the manufacturer. This evaluation protocol is intended to provide an abbreviated, but sufficient, characterization of the instrument to assess CW agent identification capabilities and aid the user in the selection of detection equipment for CW agent detection.

#### PROCEDURE FOR BG ANALYSIS

Another objective of this evaluation is to determine the ability of the instrument to identify biological samples by placing 0.5 mg or more of talc powder or fine dust mixed with BG spores (the bio-agent simulant) on the diamond sensor. Make mixtures with 1%, 10%, 25%, 50%, and up to 75% (by weight) to the point where positive identification is achieved. Ensure contact of the sample with the diamond by applying pressure with the flat end of the stainless steel rod, included with the instrument. Apply pressure to "7" on the LED display. Observe the sample on the LCD video monitor as pressure is applied. Collect the spectrum. While the spectrum is displayed on the computer monitor, use the cursor to locate absorption bands indicative of protein. Absorption bands near  $3300\text{ cm}^{-1}$  (NH),  $1640\text{ cm}^{-1}$  (amide I),  $1530\text{ cm}^{-1}$  (amide II), and  $1250\text{ cm}^{-1}$  (amide III) indicate protein and therefore, biological material. Further confirmation of biological identification is provided by a spectral search against the reference library. Note the results. Repeat for each mixture.

The bio-simulant testing procedural protocol is to investigate whether or not the TravelIR instrument can detect biological spores, using *Bacillus subtilis* var. *niger* (BG) as a test organism, and to provide preliminary data regarding response and sensitivity in the presence of a sample matrix.

The CWA and Bio-simulant testing followed similar procedures outlined below to prepare the test matrices. The percentage concentration can be weight to weight, weight to volume, or volume to volume. Therefore, the concentration numbers appearing in this report should be treated as approximate at those percentages due to the minute amount of sample used for the serial dilutions and different physical forms of the matrices (i.e., solid or liquid).

## SAMPLE PREPARATION

Samples will be prepared by serial dilutions. TravelIR requires very small sample per analysis. Minimum quantity practically required for the experiment will be used to minimize waste. Samples will be analyzed as follow:

1. Blank instrument according to manufacturer's instructions
2. Test triplicate samples of a single preparation. Triplicate samples were tested to account for possible inconsistencies of sample homogeneity.
  - a. Place sample into sample cell per manufacturer's instructions and place into TravelIR device
  - b. Test per manufacturer protocol
  - c. Clean apparatus and sample cell as recommended by manufacturer
  - d. Repeat steps 2.a through 2.c with second sample
  - e. Repeat steps 2.a through 2.c with third sample
  - f. Analyze results. Check for reproducibility of signals and signal strengths.
3. Prepare serial dilutions of sample in matrix
  - a. Weigh (solid) or measure (liquid) appropriate amount of sample
  - b. Weigh or measure the amount of matrix and add to sample tube giving a 50% sample concentration ("1:1" dilution)
  - c. Mix well using a vortex, bead beater or other apparatus for at least two minutes after materials appear well blended.
  - d. Weigh or measure an amount of 50% sample dilution from 3.b
  - e. Weigh or measure same amount of matrix and add to above 3.d sample tube to yield a 25% sample concentration
  - f. Mix well using a vortex, bead beater or other apparatus
  - g. Continue preparing serial 1:1 dilutions to yield the respective samples in the test matrix

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## APPENDIX C

### DATA TABLES AND PLOT FIGURES

#### **Results of Testing BG Spores Mixed in Different Substrates, From Each of the Individual Replicates Tested, From Each of the Five Matrices Tested.**

The following tables and figures represent the respective quality score values derived from the test samples at the respective concentration following the routine subtraction protocol. The quality score values were extended to include the quality threshold value down to zero in lieu of the quality value of >80 to obtain the top twenty identified compounds. Any hit of "neat BG" of any quality value was considered a positive result for the purpose of this report.

Because of the widely scattered points, the averaged value was calculated and represented as the dashed line through the points.

Table C - 1. SpectralID Quality Scores of BG Diluted in Silica

BG Concentration, %	Quality Values of Trials			
	1	2	3	AVG
50.00	91.90	87.60	90.90	90.13
25.00	89.16	74.80	73.30	89.16
12.50	73.79	77.31	*	75.55
6.25	76.76	55.86	67.22	66.61
3.13	63.80	56.01	59.05	59.62
1.56	62.14	57.82	57.86	59.27
0.78	*	56.19	56.50	56.35
0.39	48.52	*	*	48.52
0.20	52.06	47.31	50.18	49.85

\*Data not available

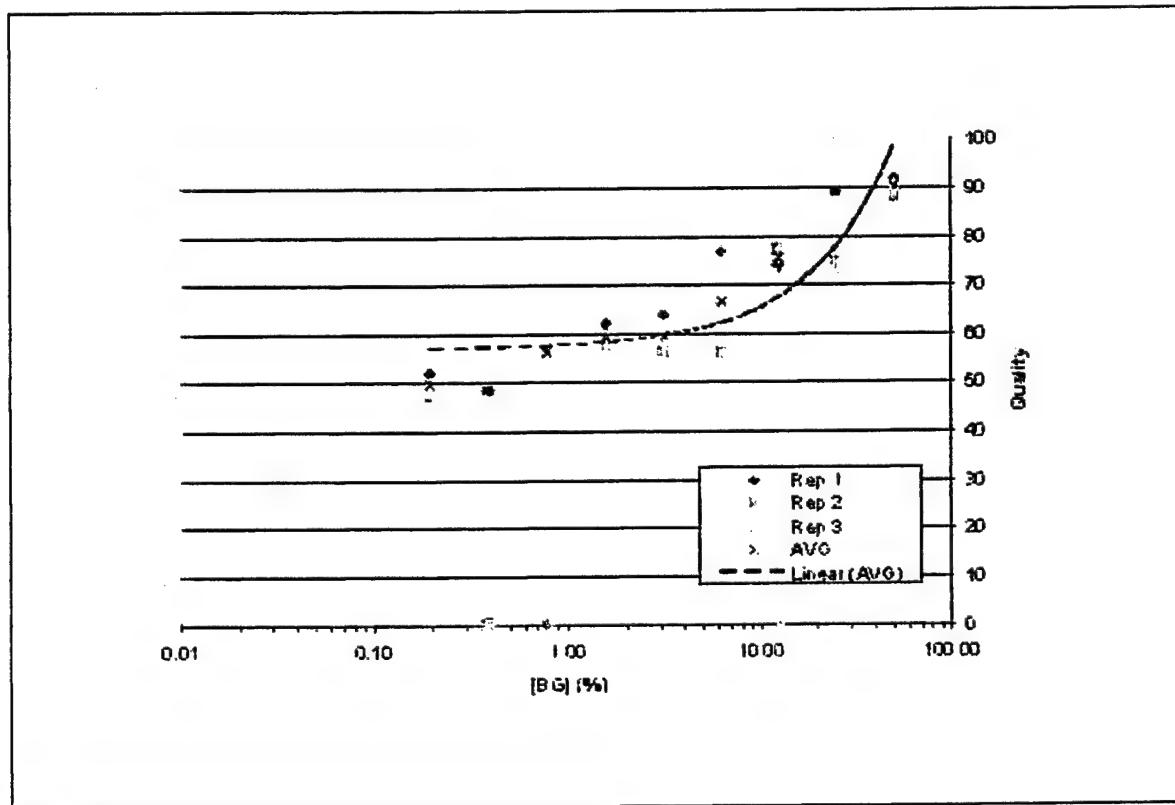


Figure C - 1. SpectralID Quality Scores of BG Diluted in Silica

Table C - 2. SpectralID Quality Scores of BG Diluted in Non-Fat Dry Milk

BG Concentration, %	Quality Values of Trials			
	1	2	3	AVG
0.78	75.00	75.40	75.30	75.23
0.39	54.71	73.70	74.40	67.60
0.20	73.70	26.61	74.20	58.17
0.10	39.20	61.65	73.60	58.15
0.05	32.79	6.54	7.14	15.49
0.02	0.94	69.70	1.61	24.09

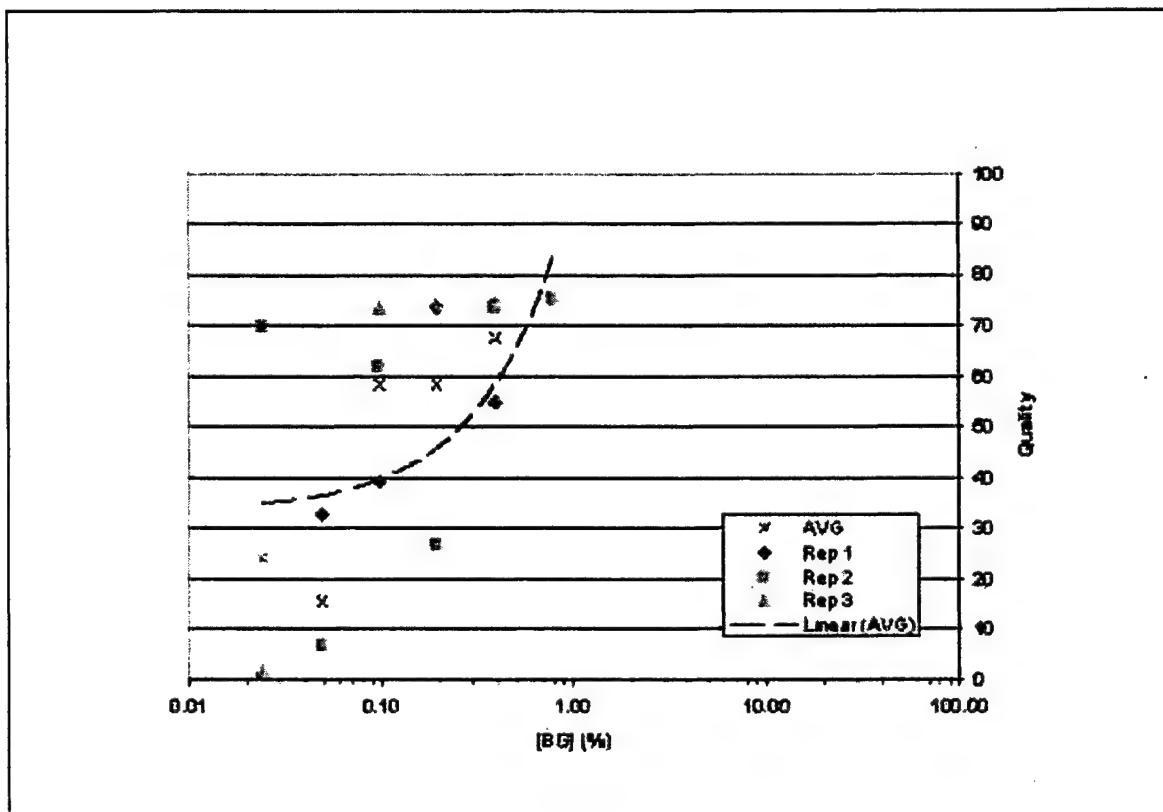


Figure C - 2. SpectralID Quality Scores of BG Diluted in Non-Fat Dry Milk

Table C - 3. SpectralID Quality Scores of BG Diluted in Floor Wax

BG Concentration, %	Quality Values of Trials			
	1	2	3	AVG
25.00	93.10	95.10	94.90	94.37
12.50	74.00	69.03	56.99	66.67
6.25	44.87	47.60	44.34	45.60
3.13	26.82	48.86	13.11	29.60
1.56	0.00	1.57	2.63	1.40
0.78	0.00	0.00	8.18	2.73
0.39	0.00	0.25	0.00	0.08

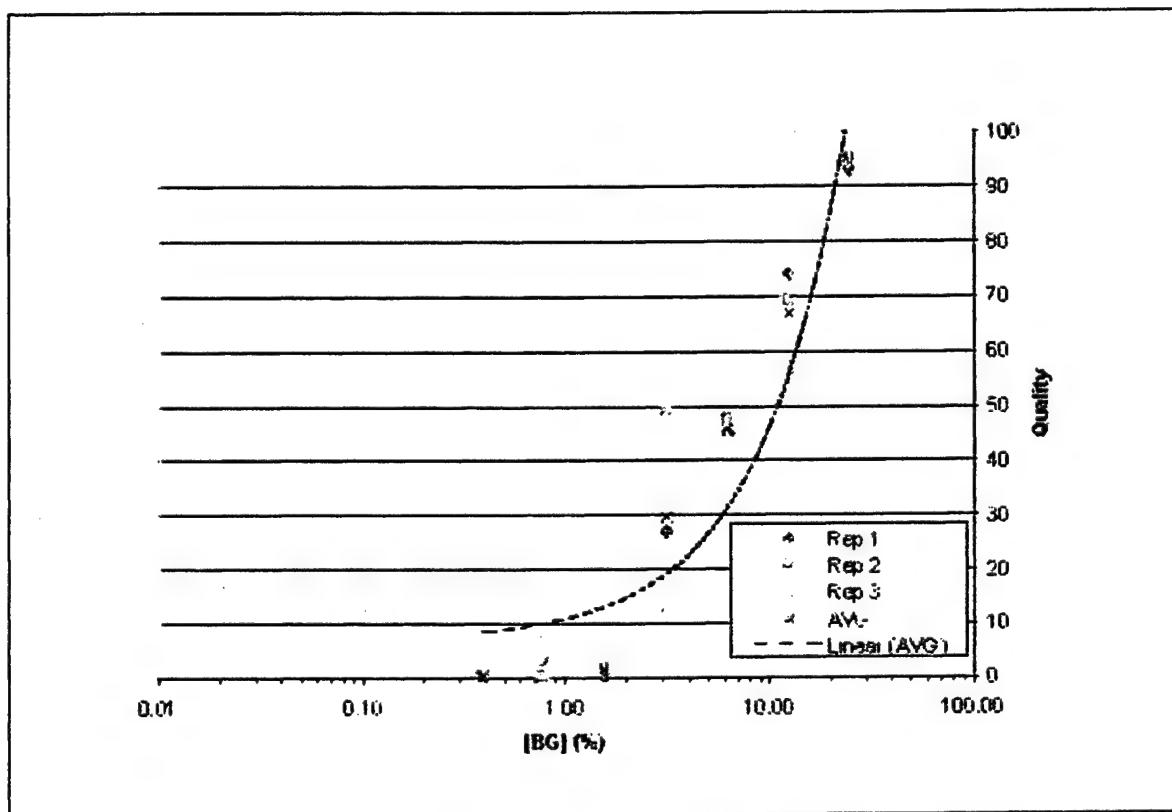


Figure C - 3. SpectralID Quality Scores of BG Diluted in Floor Wax

Table C - 4. SpectralID Quality Scores of BG Diluted in Aqueous Film Forming Foam (AFFF)

BG Concentration, %	Quality Values of Trials			
	1	2	3	AVG
50.00	84.80	85.00	83.70	84.50
25.00	78.05	72.27	60.65	70.32
12.50	14.30	56.18	37.77	36.08
6.25	29.01	26.37	36.87	30.75
3.13	45.40	47.28	49.42	47.37
1.56	43.00	36.98	18.70	32.89
0.78	27.10	18.80	24.42	23.44
0.39	39.88	28.20	6.18	24.75
0.20	22.56	28.98	19.31	23.62
0.10	27.26	33.69	28.43	29.79
0.05	52.73	44.52	47.54	48.26
0.02	22.33	37.11	37.93	32.46

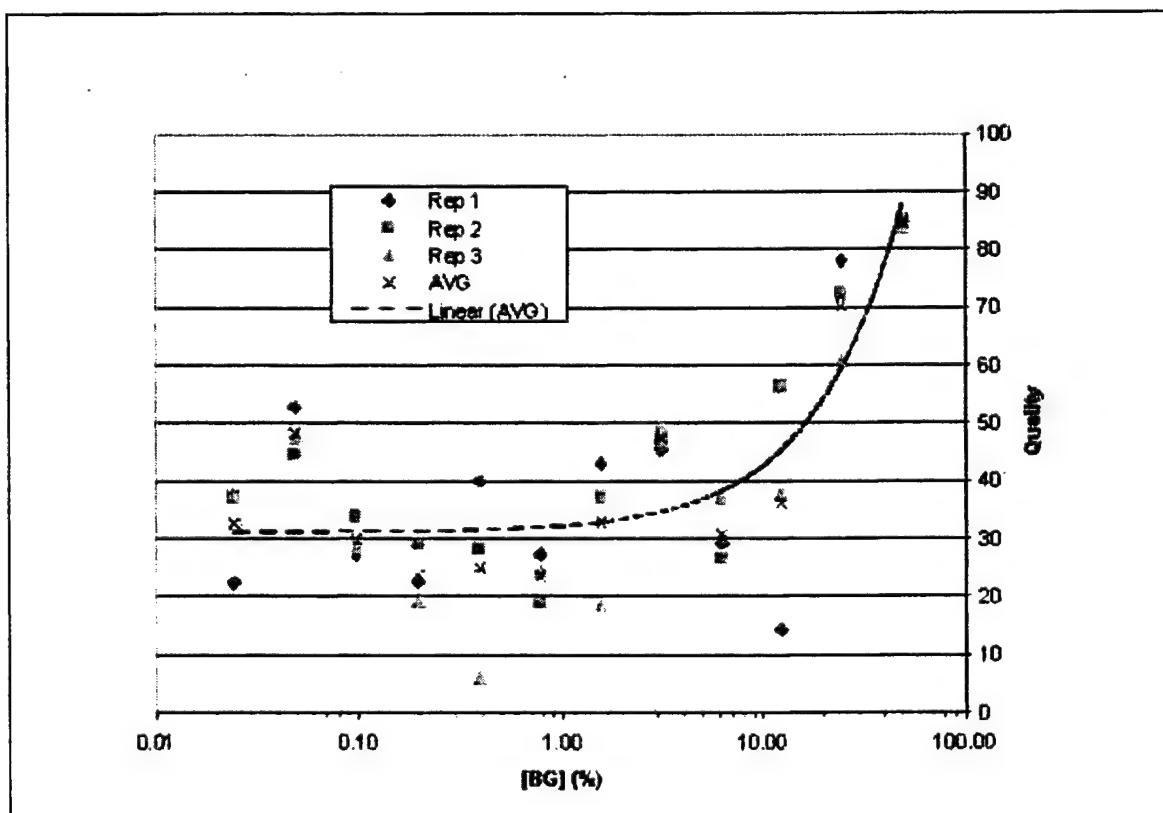


Figure C - 4. SpectralID Quality Scores of BG Diluted in AFFF

Table C - 5. SpectralID Quality Scores of BG Diluted in Diesel Fuel

BG Concentration, %	Quality Values of Trials			
	1	2	3	AVG
50.00	97.30	98.50	99.00	98.27
25.00	79.30	75.50	64.50	73.10
12.50	71.16	91.65	83.09	81.96
6.25	88.77	63.10	90.42	80.76
3.13	60.42	88.13	84.92	77.82
1.56	87.83	90.12	79.56	85.84
0.78	67.22	24.47	30.32	40.67
0.39	1.91	14.47	50.74	22.37

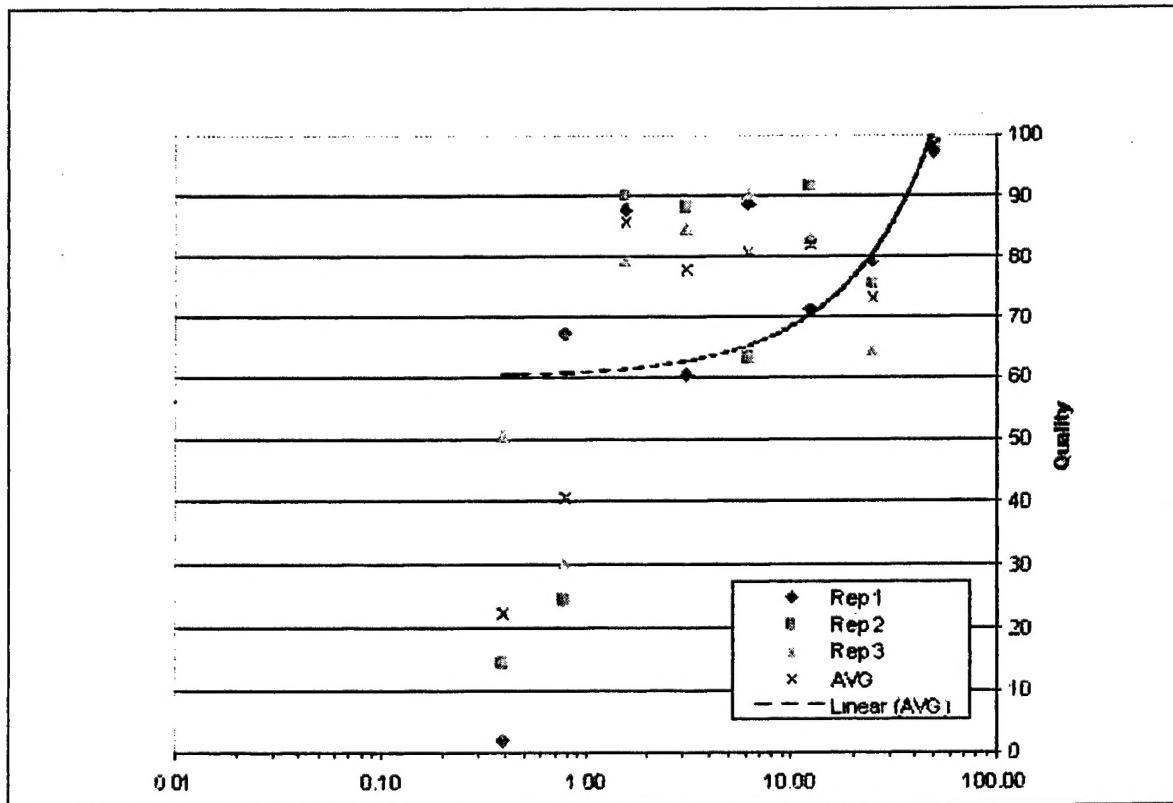


Figure C - 5. SpectralID Quality Scores of BG Diluted in Diesel

## APPENDIX D

### SIMPLE EXTRACTION PROCEDURE FOR THE INFRARED ATR SPECTROMETRIC ANALYSIS OF MIXTURES CONTAINING CW AGENT

#### Mixture extraction kit:

15 x 45 mm, 1/8 oz volume sample vials with Teflon coated inner cap

Distilled water

Spectrophotometric grade chloroform

Vials are pre-filled with 0.5 mL solvent

Micro-spatula

5 ¼ in. disposable Pasteur pipet

#### Supplementary Materials:

TravelIR User's Guide

TravelIR QualID Application Software User's Guide

#### Extraction of Solid Samples

1. Place two vials on working area, one containing water and the other containing chloroform ( $\text{CHCl}_3$ ).
2. Using a micro-spatula, place approximately 100 mg (0.1 g) of the solid sample into each vial.
3. Secure the cap on each vial and shake each vial vigorously for 1 min.
4. Allow the solid material to collect at the bottom of each vial.
5. Note the solubility of the solid material in each solvent.
6. If the solid material is insoluble in chloroform, the chloroform solution will be the working solution.
7. If the solid material is soluble in chloroform but insoluble in water, the aqueous solution will be the working solution.
8. Ensure that the diamond ATR sensor is clean and collect a background.
9. Place a 5  $\mu\text{L}$  aliquot of the working solution onto the diamond ATR sensor of the TravelIR HCl portable FT-IR system.
10. If the solution is a chloroform solution, cover the sensor and solution with the volatiles cover.
11. Press *Sample Analysis* and monitor the real-time spectral display, observing the solvent absorption bands.
12. If the working solution is an aqueous solution, cover the diamond ATR sensor with the volatiles cover as soon as the water evaporates (the solvent bands have minimized).
13. When the solvent absorption bands have minimized after removing the volatiles cover and the solute spectrum is clearly observed, begin the spectral data collection by pressing *Start Analysis*.

14. The IR spectrum of the solute(s) will then be recorded and compared against the selected databases.

Figure D-1 shows that GB can be extracted from a solid substrate (silica) to yield a detection and identification. GB is mixed with silica. The solid-liquid mixture is extracted with chloroform. The chloroform extract is analyzed with the TravelIR HCl portable FT-IR spectrometer.

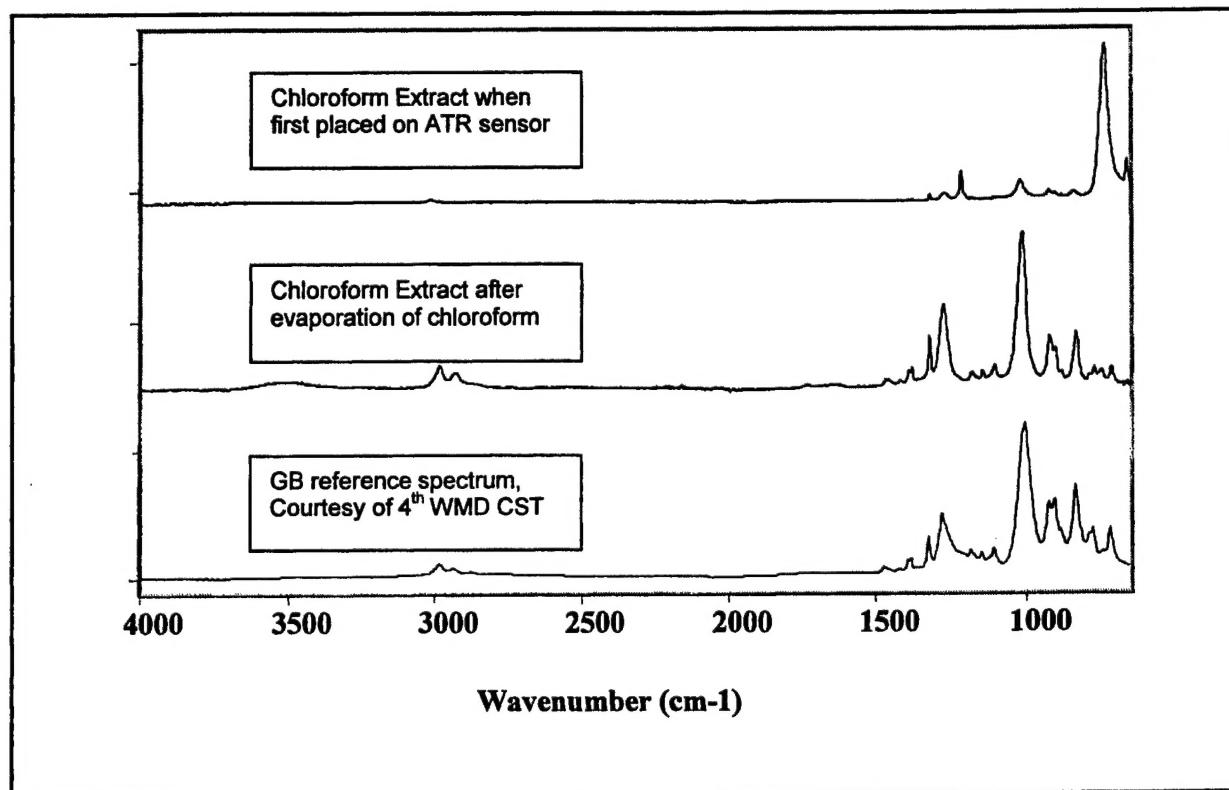


Figure D - 1. Spectra of the Extraction of a Solid-Liquid Mixture Sample (GB in Silica)

#### Extraction of Liquid Samples

1. Place two vials on working area, one containing water and the other chloroform ( $\text{CHCl}_3$ ).
2. Using a  $5\frac{1}{4}$ " disposable Pasteur pipet, place several (3) drops of the liquid sample into each vial.
3. Secure the cap on each vial and shake each vial vigorously for 1 minute.
4. Allow the liquid phases to separate.
5. Note the solubility of the liquid sample in each solvent.
6. If the liquid sample is insoluble in chloroform, the chloroform solution will be the working solution. The density of chloroform is 1.484; therefore, the chloroform phase

will be at the bottom when mixed with an aqueous phase. Sample the bottom layer when the chloroform solution is the working solution.

7. If the liquid sample is soluble in chloroform but insoluble in water, the aqueous solution will be the working solution.
8. Ensure that the diamond ATR sensor is clean and collect a background.
9. Place a 5  $\mu$ L aliquot of the working solution onto the diamond ATR sensor of the TravelIR HCl portable FT-IR system.
10. If the solution is a chloroform solution, cover the sensor and solution with the volatiles cover.
11. Press *Sample Analysis* and monitor the real-time spectral display, observing the solvent absorption bands.
12. If the working solution is an aqueous solution, cover the diamond ATR sensor with the volatiles cover as soon as the water evaporates (the solvent bands have minimized).
13. When the solvent absorption bands have minimized after removing the volatiles cover and the solute spectrum is clearly observed, begin the spectral data collection by pressing *Start Analysis*.
14. The IR spectrum of the solute(s) will then be recorded and compared against the selected databases.

Figure D-2 is an example extraction spectrum of the chloroform layer. VX is mixed with an aqueous emulsion, liquid floor wax product. The liquid mixture is extracted with chloroform. The chloroform extract is analyzed with the TravelIR HCl™ portable FT-IR spectrometer.

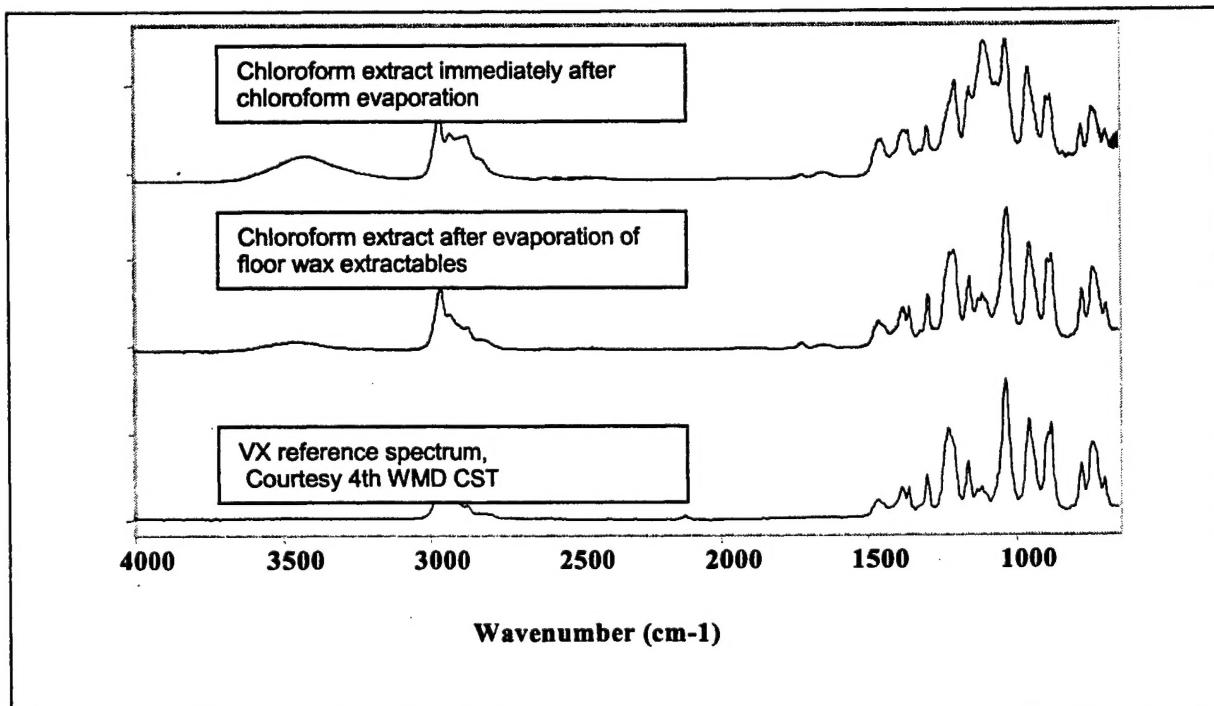


Figure D - 2. Spectra of the Extraction of a Liquid Mixture Sample (VX in Floor Wax)